

The relationship of Heat Shock Protein-70(HSP-70) and toxoplasmosis among women with abortion

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Abstract: Toxoplasmosis is a wide spread disease in our society, moreover it plays an important role in causation of abortion in pregnant women. The present study was carried out to evaluate the role of Heat Shock Protein-70(HSP-70) in the immune response against infection with *Toxoplasma gondii* among women with abortion.

The present study was carried out on(35) women ,all of them having abortion and proved by confirmatory tests that they have an active form of toxoplasma infection. It also included twenty apparently healthy women as a control group . The patients were between 15 to 50 years of age, blood samples were collected from them during the period between May 2010 to May 2011. The Cytokines that were measured among studied groups by ELISA technique included HSP-70, Interleukin-10(IL10) , Gamma Interferon(IFN γ) and Interleukin-12(IL-12), thereafter cytokines were remeasured after receiving complete course of treatment.

The study showed a high significant increase in serum level of HSP-70 in patients with IgM seropositivity (4.138pg/ml) when compared with serum levels in control and post treated groups (1.990pg/ml , 1.015pg/ml) respectively. It was also found that there was a concomitant increase in serum level of HSP-70 and serum level of both IFN γ and IL-12 in the early stage of acute infection (19.023, 81.292) respectively.

الخلاصة:

مرض داء المقوسات من الامراض الواسعة الانتشار في مجتمعاتنا ، علاوة على كونه يلعب دورا مهما في احداث الاسقاط لدى النساء الحوامل. اجريت هذه الدراسة لمعرفة دور Heat Shock Protein-70 (HSP-70) في الاستجابة المناعية ضد هذا المرض لدى النساء اللاتي عانين اسقاطا.

تضمنت تلك الدراسة جمع عينات من دم (35) من اولئك المرضى والذي ثبت مختبريا اصابتهن بهذا المرض كما تم جمع عينات من (20) من النساء الاصحاء كمجموعه ضابطه، تراوحت اعمار من شملتهم تلك الدراسة ما بين 15-50 سنة وللفترة ما بين ايارس 2010 و ايارس 2011 وقد تم قياس مستوى HSP-70 في مصل اولئك المرضى والمجموعه الضابطه بالاضافه الى قياس مستوى الساييتوكاين العاشر (Interleukin-10)، الساييتوكاين الثاني عشر (IL-12) والكاما انتفيرون (Gamma Interfeon, IFN γ) واخيرا تم قياسهن بعد اخذ العلاج اللازم.

اظهرت نتائج تلك الدراسة زياده وبصوره معنويه في مستوى ال (HSP-70) في دم اولئك المرضى (4.138 pg/ml) في المراحل المبكره من الاصابه عند مقارنتها لمستواه في المجموعه الضابطه ومجموعه المرضى بعد اخذهم العلاج اللازم (1.990pg/ml , 1.015 pg/ml) وعلى التوالي، كما تبين بان الزيادة في مستوى HSP-70 ترافق مع الزيادة الحاصله في مستوى (IFN γ & IL-12) في المراحل المبكره للمرض (19.023 pg/ml ; 81.2 pg/ml) وعلى التوالي.

INTRODUCTION:

Heat shock proteins (HSPs) was used first to describe *Drosophila melanogaster* proteins expressed at elevated temperatures (1). It is a collective term for a number of ubiquitously expressed and highly conserved cytoprotective eukaryotic and bacterial proteins. In 1962, Ritossa reported that heat and the metabolic dinitrophenol induced a characteristic pattern of puffing in the chromosomes of *Drosophila*, (2 ; 3).

This discovery finally led to the identification of the heat-shock proteins (HSP) or stress proteins . They are named according to their molecular weight. For example, Hsp60, Hsp70

and Hsp90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70 and 90 kilodaltons in size, respectively (4).

The intracellular role of heat shock proteins is that of molecular chaperones. These proteins stabilize unstable proteins and leading to correct folding, assembly of proteins into oligomeric structures, transport to subcellular compartments, or controlled switching between active and inactive conformational states (5).

These proteins are found in the circulation of normal subjects (6) and their concentrations decline with age (7). Altered serum levels of these proteins have been found in association with certain disease states (8; 9; 10). Antibodies against various heat shock proteins are also observed in normal subjects (6) and various disease states (11; 12; 13; 14; 15).

Concerning Heat Shock Protein (HSP-70), it plays an important role in transmembrane transport of proteins, by stabilizing them in a partially-folded state. Hsp70 proteins can act to protect cells from thermal or oxidative stress. These stresses cause partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially-denatured proteins from aggregating, and allows them to refold (16).

Heat Shock Protein-70 (Hsp70) seems to be able to participate in disposal of damaged or defective proteins. (17). In addition to improving overall protein integrity, Hsp 70 directly inhibits apoptosis, Hsp 70 inhibits this process by blocking the recruitment of procaspase-9 to the Apoptosis Activating Factor-1(Apaf-1)/dATP/cytochrome c apoptosome complex (18).

MATERIALS AND METHODS:

Collection of Blood Samples:

Thirty five blood samples were collected from women with active form of toxoplasmosis after confirmation with IgM test, the age was ranged between 16 to 45 years old from different health centers in Najaf governorate), five ml of venous blood was drawn using a 5 ml size disposable syringe then transferred to 10 ml disposable sterile serum tube. The blood samples were left to clot then centrifuged at 3000 rpm for 5 minutes to separate the serum. Serum samples transferred to eppendorf tubes and stored at 4 - 8 °C for 24 hrs. If long period of storage is required, the sera will be kept frozen at - 20 °C until use.

ELISA tests:

The following tests were done according to manufacturer's instructions:

- 1-IgG and IgM ELISA tests. (Biocheck/USA)
- 2-Heat Shock Protein-70 (HSP-70). (USBiological)
- 3-Gamma Interferon (IFN- γ).(Immunotech/France)
- 4- Interleukin-10 (IL-10). (USBiologica/USA)
- 5- Interleukin-12 (IL-12). (USBiologica/USA)

RESULTS:

Heat Shock Protein-70 (Hsp-70):

There was a statistically significant difference ($P < 0.000$) between serum level of HSP-70 in patients with acute infection (displaying positive IgM antitoxoplasma antibody) (4.138 ± 1.05847) and serum level in control and post treatment group (1.015 ± 0.630 , 1.990 ± 0.961) respectively and there was no significant difference between serum level in

acute state with positive both IgG and IgM antitoxoplasma antibodies (1.1017 ± 0.568) and the level in control and post treated groups. (Table 1)

Table (1) : Serume hsp-70 levels in study groups.

Cytokine type		Mean concentration \pm SD of Hsp-70 No.(Conc. Pg/ml)
Acute cases	With positive IgM only	4.138 \pm 1.05847*
	With positive IgM/IgG	1.1017 \pm 0.568**
	Total	3.271 \pm 1.677***
Post treatment		1.990 \pm 0.961* ,**
Control group		1.015 \pm 0.630***
P value		Paired t-test=7.771 p value=0.000* Paired t-test=1.421 p value=0.189** Independent t test=5.768 pvalue=0.000***

*paired t-test between acute (IgM only) group & post treatment group.

** paired t-test between acute (IgM/IgG) group & post treatment group.

***Independent t-test between acute group & control group.

IFN γ Level:

A high significant increase ($p < 0.000$) of serum IFN γ was recorded in acute cases with only positive IgM antitoxoplasma antibody (19.023 ± 2.881 pg/) when compared with post treatment and control serum level (3.052 ± 1.416 , 2.211 ± 1.193) ,whereas there was no significant difference between serum level of this cytokine in acute cases with both positive IgG and IgM antitoxoplasma antibodies and serum level in post treatment and control groups ($P = 0.689$) table figure (1).

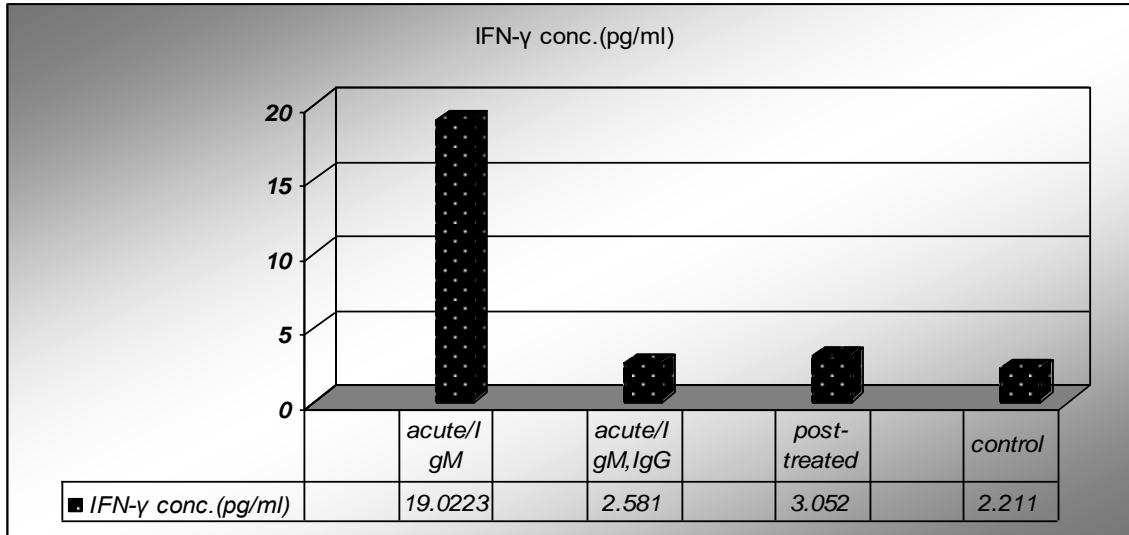


Figure (1): mean of serum IFN Gamma level in different study groups.

Note: *paired t-test between acute (IgM only) group & post treatment group= **14.256;P=0.000**.

** paired t-test between acute (IgM/IgG) group & post treatment group=**0.392;P=0.698**.

***Independent t-test between acute group & control group **P=0.006**.

IL-12 Level.

There was a high significant increase of IL-12 levels (P=0.000) in the sera of patients with only IgM seropositive toxoplasmosis (81.292 ± 29.278) when compared to that of control group and post treatment level, whereas level among those with positive IgG and IgM antitoxoplasma antibodies (15.311 ± 17.595) was statistically lower than post treated group (P=0.000). The mean serum level of IL-12 among acute cases was not different statistically from level registered among control group P=0.16. (Figure 2)

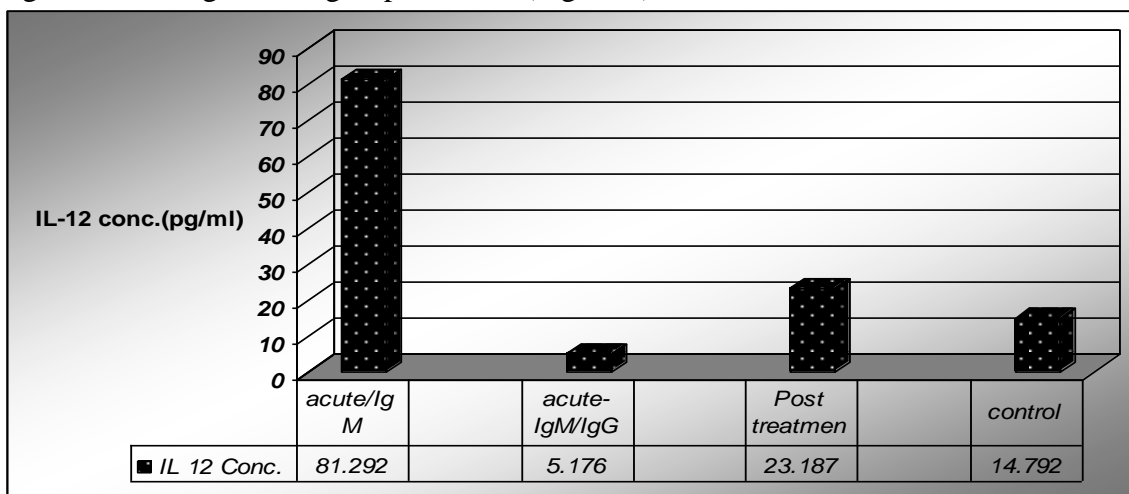


Figure (2): mean of serum IL-12 in different study groups.

Note: *paired t-test between acute (IgM only) group & post treatment group=**6.46 ;P value=0.00**.

** paired t-test between acute (IgM/IgG) group & post treatment group.=**12.462; P value=0.000**.

***Independent t-test between acute group & control group=**1.417;Pvalue=0.16**.

HSP-70/IFN γ ratio.

The study revealed that high production of hSP-70 with concomitant increase of IFN γ serum level in patient with IgM seropositive group ,consequently resulting in no significant difference in ratio ($P>0.05$) of HSP-70:IFN γ (0.217) among patients in the early stage of disease when compared with the ratio for the controlled group (0.45) . (Table-2)

Table (2): The ratio of hsp-70/IFN γ in sera of patients with toxoplasmosis compared with healthy control group

Groups	Mean of hsp-70 (Pg/ml)	Mean of IFN γ (Pg/ml)	Hsp-70/IFN γ ratio
Control	1.015 \pm 0.630	2.211 \pm 1.193	0.45
Patients	4.138 \pm 1.05847	19.023 \pm 1.107	0.217
P value	P value >0.05		

IL-10 Level.

It was obvious that there was a high significant increase in IL-10 level in the sera of patients with acute toxoplasmosis having both positive IgG and IgM (132.258 \pm 97.488pg/ml) when compared with control and post treated groups (5.331 \pm 1.312pg/ml, 6.394 \pm 1.591pg/ml). The same was applied in acute cases with only positive IgM (14.942 \pm 3.539pg/ml) where serum level was significantly higher than other studied groups ($P=0.000$). (Figure 3)

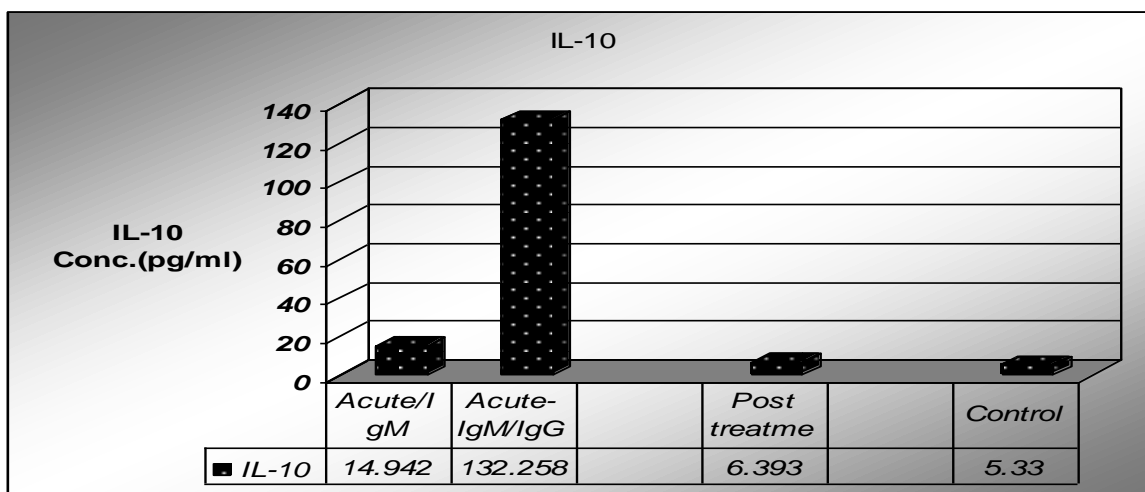


Figure (3): mean of serum IL-10 in different study groups.

Note:*paired t-test between acute (IgM only) group & post treatment group= 7.211 ; P value= 0.000 .

** paired t-test between acute (IgM/IgG) group & post treatment group= 6.488 P value= 0.000 .

***Independent t-test between acute group & control group= 4.245 pvalue= 0.000 .

DISCUSSION:

A high level of serum concentration of HSP-70 was recorded among patients with ELISA seropositive of IgM antitoxoplasma antibody (4.138pg/ml) and there was a significant difference between these level and level in post treated and control groups, whereas serum level in patient with seropositivity with both IgM&IgG had no significant difference from post treated group.

The present finding was inconsistent with result found by (19),who concluded that HSP70 have an inhibitory effect on the induction of nitric oxide (NO) release by peritoneal macrophages of *T.gondii*-infected mice .Their findings identify HSP70 was danger and lethal signal during acute *T.gondii* infection , (19) also concluded that a decrease in NO release in *T.gondii*-infected was correlated with increased parasite replication and decreased cyst formation. They also found that that there was a reverse relation between HSP70 and IFN γ level and this finding is incompatible with the finding of present study, HSP70 induce active replications and thereby bradyzoites to tachyzoites conversion,the reverse will happen in case of high serum level of IFN γ where these interferon will enhance tachyzoites to bradyzoites conversion with consequent cyst formation.

However, the current finding was compatible with a more recent finding by (20) who found that HSP70 induce maturation of bone marrow-derived dendritic cells,they also demonstrated that IL12 production was enhanced from HSP70-stimulated bone marrow-derived dendritic cells with cosequent enhancing of IFN γ production ,so there was correlation between T-Helper-1(Th-1) cytokine concentrations and HSP70 serum level.

Dendritic cells (DCs) are bone-derived professional antigen-presenting cells (APC) capable of activating naïve antigen-specific T cells and initiating adaptive protective immunity (21). Dendritic cells maturation is important to induce T cell activation that plays a central role in protective cellular immunity against intracellular protozoan infection.In peripheral tissues DCs are found in immature form that are highly effective in acquiring and processing antigen (22).Dendritic cells ,when exposed to microbial infection , will migrate to draining lymph nodes ,then they will mature into highly potent APCs by up-regulating the expression of the major histocompatibility complex (MHC) class I and classII molecules and T cell-stimulatory surface molecules such as CD40,CD80 and CD86 (23), mature DCs present antigens as processed peptides to naïve CD4 and CD8 cells in drainig lymph nodes (24) and (25) .

HSP70-stimulated DCs were capable to produce IL-12 via Toll-like receptor 4(TLR4) /myloid differentiation factor88(MyD88) thereby modulate T-helper polarization toward T-helper type-1 ,this conclusion was compatible with the present result,where there was high level of HSP70 ,with T-Helper-1(Th1) bias of immune response so the present study revealed a concomitant increase of Th1 cytokines with the increase of HSP70.

Some researchers found that there will be lack of IL-12 production from HSP70-stimulated DCs of MyD88-deficient mice which will end in T-Helper-2(Th2) dominance (26; 27) . (28) and (29) reported that a combination of TLR2 and TLR4 with CD14 induced the MyD88-independent synergistic augmentation of HSP70-induced proinflammatory cytokine production ,suggesting that HSP70 might signal by both MyD88-dependent and MyD88-independent signal pathways .

Regarding to HSP70:IFN γ ratio ,it was obviously that there was no prominent difference in ratio among patients in early acute stage of disease and ratio among control group ,indicating a concomitant increase of both HSP70 and IFN γ ,the result was compatible with above

conclusion about the pattern of increase of HSP70 and Th1 cytokine, however no similar research mentioned or measured such a ratio.

In comparison with present results there was no published data, available about relationship of HSP-70 or other heat shock proteins with *T.gondii* infection, in Iraq, Arabian or Islamic countries to compare our results. However these findings will probably open new era for researches, in Iraq and nearby countries, concerning relationship between HSP70 and other heat shock proteins, not only with *Toxoplasma* infection but with other infectious and non infectious diseases, moreover the researches in developed countries was not so much about this subject and its relation with many diseases so the current subject is interesting one and containing many debatable informations that is needed to be clarified by further researches.

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